Seasonal patterns of polycyclic aromatic hydrocarbons in digestive gland and arm of octopus (*Octopus vulgaris*) from the Northwest Atlantic

Miguel Semedo, Marta Oliveira, Filipa Gomes, Maria Armanda Reis-Henriques, Cristina Delerue-Matos, Simone Morais, Marta Ferreira

ABSTRACT

Among organic pollutants existing in coastal areas, polycyclic aromatic hydrocarbons (PAHs) are of great concern due to their ubiquity and carcinogenic potential. The aim of this study was to evaluate the seasonal patterns of PAHs in the digestive gland and arm of the common octopus (*Octopus vulgaris*) from the Northwest Atlantic Portuguese coast. In the different seasons, 18 PAHs were determined and the detoxification capacity of the spe- cies was evaluated. Ethoxyresorufin O-deethylase (EROD) and ethoxycoumarin O-deethylase (ECOD) activities were measured to assess phase I biotransformation capacity. Individual PAH ratios were used for major source (pyrolytic/petrogenic) analysis. Risks for human consumption were determined by the total toxicity equivalence approach. Generally, low levels of PAHs were detected in the digestive gland and in the arm of octopus, with a predominance of low molecular over high molecular weight compounds. PAHs exhibited seasonality in the con- centrations detected and in their main emission sources. In the digestive gland, the highest total PAH levels were observed in autumn possibly related to fat availability in the ecosystem and food intake. The lack of PAH elimina- tion observed in the digestive gland after captivity could be possibly associated to a low biotransformation capac- ity, consistent with the negligible/undetected levels of EROD and ECOD activity in the different seasons. The emission sources of PAHs found in the digestive gland varied from a petrogenic profile observed in winter to a pyrolytic pattern in spring. In the arm, the highest PAH contents were observed in June; nevertheless, levels were always below the regulatory limits established for food consumption. The carcinogenic potential calculated for all the sampling periods in the arm were markedly lower than the ones found in various aquatic species from different marine environments. The results presented in this study give relevant baseline data for environmental monitoring of organic pollution in coastal areas.

Keywords

Octopus, Polycyclic aromatic hydrocarbons, Source analysis, Biotransformation, Total toxicity equivalence

1. Introduction

The aquatic environment is continuously exposed to a wide range of chemicals that are released by anthropogenic activities including polycyclic aromatic hydrocarbons (PAHs) which are of great importance due to their ubiquitous presence in the environment and the carcinogenic potential of some of them. The U.S. Environmental Protection Agency (USEPA) listed 16 priority PAH compounds (USEPA, 1986) while the European Commission listed 15 PAHs to be monitored in foodstuff (Commission, 2006). Recently 4 PAHs (benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene) were determined as the most appropriate indicators for the presence of carcinogenic and genotoxic PAHs in foodstuff (Commission, 2011). Benzo(a)pyrene is classified by the International Agency for Research on Cancer (IARC) as a known carcinogenic to humans (group 1) whereas other

PAHs are considered as probable (2A – dibenzo(a,l)pyrene and dibenz (a,h)anthracene) or possible carcinogens (2B – naphthalene, benz(a) anthracene, chrysene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene and indeno(1,2,3-cd)pyrene) (IARC, 2010). Besides this classification dibenzo(a,l)pyrene has been considered the most potent carcinogen among the PAHs (Dreij et al., 2002; Okona-Mensah et al., 2005; Shou et al., 1996). The association between PAH levels in tissues of aquatic organisms and genotoxic effects have been reported in previous studies (Santos et al., 2010; Skarphéðinsdóttir et al., 2007). Although PAHs can enter the aquatic environment from natural sources (e.g. volcanic eruptions, forest fires, oil leaks, etc.) anthropogenic sources are the main causes for PAH environmental contamination (Bucheli et al., 2004; Slezakova et al., 2013; Yunker et al., 2002). PAHs may enter the marine environment by river runoff, atmospheric inputs or wet deposition of petroleum or petroleum products (Wolska et al., 2011). PAHs have been measured in surface water in levels ranging from 113 to 459 ng/L (Valavanidis et al., 2008), in sediments with levels around 200 ng/g d.w. (Boitsov et al., 2009) and aquatic organisms with concentrations reaching 69.2 μg/kg w.w. (Lima et al., 2008) in various monitoring studies for aquatic pollution. PAHs in the aquatic environment can be derived from various sources: 1) pyrolytic PAHs produced by the incomplete combustion of organic material (e.g. coal and wood burning, petrol and diesel combustion, industry) generating mainly higher molecular weight compounds; 2) petrogenic PAHs mainly originating from crude oil spills (or refined products from petroleum industries) and their composition is often influenced by lighter compounds; 3) biogenic PAHs formed from recent biological processes such as microbial activity and diagenetic processes, and degradation of organic matter (Boitsov et al., 2009; Tobiszewski and Namieśnik, 2012; Webster et al., 2002). PAHs are emitted as mixtures and the relative concentrations of certain PAHs may help distinguish the emission sources. Different PAH ratios have been used to identify emission sources involving usually pairs of PAHs with same molar mass and similar physicochemical properties so they undergo similar environmental fate processes (Bucheli et al., 2004; Francioni et al., 2007; Tobiszewski and Namieśnik, 2012; Yunker et al., 2002). Aquatic organisms might accumulate PAHs through food, water and sediments (Costa et al., 2011; Nakata et al., 2003). Concerning cephalopods some studies evaluated the levels of PAHs in squids (Gomes et al., 2013; Martí-Cid et al., 2008; Nakata et al., 2003; Perugini et al., 2007; Unger et al., 2008) nevertheless no data was found in the literature concerning PAH levels in the digestive gland and arm of *Octopus vulgaris* which make this research an important baseline study. This species combines many characteristics needed in a sentinel species: it is abundant and easy to catch, has a short life span and is an opportunistic predator with a restrict home range providing useful information about the habitat and local pollutants exposure. Recently, *O. vulgaris* was proposed as a sentinel species for the presence of metals in coastal waters (Semedo et al., 2012). This new study presents: (1) the first data on levels of 18 PAHs (the 16 PAHs considered by USEPA as priority pollutants, dibenzo(a,l)pyrene and benzo(j)fluoranthene) in octopus in different seasons, (2) the evaluation of the detoxification ability of this species maintained in captivity, (3) an analysis of the PAH ratios for major source determination, and (4) the risk evaluation for human consumption concerning the PAH levels in the edible tissues. Thus, the main goal of the present study was to evaluate the seasonal patterns of individual and total PAH levels in the digestive gland and arm of *O. vulgaris* captured in the NW Portuguese coast as well as the detoxification capacity of the species.

2. Material and methods

2.1. Reagents

A reference mixture of PAHs (EPA 610) containing naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene was purchased from Supelco (Bellefonte, PA, USA). Individual standards of each compound, benzo(j)fluoranthene and dibenzo(a,l)pyrene were also from Supelco (Bellefonte, PA, USA). Working mixed standard solutions containing all the PAHs were prepared by dilution of the stock solutions with acetonitrile and stored at −20 °C. Acetonitrile was purchased from Merck (Darmstadt, Germany) and ultrapure water was obtained from a Milli-Q simplicity 185 system (Millipore, Molsheim, France). α-Dithiothreitol (99% purity), bovine serum albumin (BSA, 99% purity), resorufin sodium salt, 7-ethoxyresorufin, 7-ethoxycoumarin and 7-hydroxycoumarin were purchased from Sigma Aldrich (Steinheim, Germany). All the other chemicals were of analytical grade and purchased from local suppliers.

2.2. Study area and sampling

Common octopuses (Table 1) were collected alive by fishermen using traditional fishery traps along the NW coast of Portugal between the cities of Póvoa de Varzim and Porto (Fig. 1). The capture was performed in January, June, and November of 2011 and September of 2012 in order to comprise the four different seasons of the year. The octopuses were transported to the laboratory in a transport tank with aerated seawater in less than 2 h. In the laboratory, octopuses were separated into two groups, one group was sampled immediately (day 0) and the other placed in aerated seawater tanks (500 L; salinity 34 ± 1 PSU; temperature 19 ± 2 °C), with biological filters, for 14 days (day 14) with natural photoperiod and being fed each second day with white hake fish filets (for human consumption and purchased frozen). Nitrite and ammonium levels were maintained below 0.5 mg/L to guarantee animal welfare. Animals were euthanized after anesthesia in ice-cold water (Andrews and Tansey, 1981), measured, weighed and the digestive gland index calculated ((digestive gland mass (g) / (whole body mass (g) − digestive gland mass (g))) × 100; Table 1) according to (Otero et al., 2007). Digestive glands and arms were dissected, mechanically homogenized with a Bosch Blender (750 W, MSM7400, Slovenia) until a smooth paste was obtained and frozen at − 20 °C until chemical analysis. Parts of the digestive gland were immediately frozen in liquid nitrogen and stored at −80 °C for biochemical determinations, until further use.

2.3. Phase I biotransformation enzymes

Ethoxyresorufin O-deethylase (EROD) and ethoxycoumarin O-deethylase (ECOD) activities were measured in the microsomal fraction of the digestive glands according to Ferreira et al. (2010) and Cheah et al. (1995) with small modifications. Briefly, digestive glands were homogenized in ice-cold 100 mM potassium phosphate buffer pH 7.4, 150 mM KCl, 1 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM disodic ethylenediaminetetra acetic acid (Na_2EDTA) (Fernandes et al., 2008). Microsomes were obtained by ultracentrifugation of the 12,000 *g* supernatant at 100,000 *g* for 60 min

Table 1
Mass (g), digestive gland index (DGI) and number of octopuses (N).

		N	Mass (g)	DGI
January	Day 0	4	848.8 ± 258.0	$2.8 \pm 0.4^{a,b}$
	Day 14	4	588.8 ± 107.9	1.9 ± 0.2^{a}
June	Day 0	10	1381.2 ± 512.4	3.5 ± 0.6^{b}
	Day 14	6	857.8 ± 243.1	1.3 ± 0.4^{c}
November	Day 0	8	847.5 ± 158.9	3.7 ± 0.9^{b}
	Day 14	5	819.9 ± 78.3	2.1 ± 0.4^{a}
September	Day 0	6	740.4 ± 201.7	4.3 ± 0.9^{b}
	Day 14	3	918.1 ± 48.3	$2.9 \pm 0.3^{a,b}$
Total		46	930.1 ± 371.0	3.0 ± 1.2

Values presented as mean ± SD. Dissimilar letters denote significant differences between groups (p b 0.05).

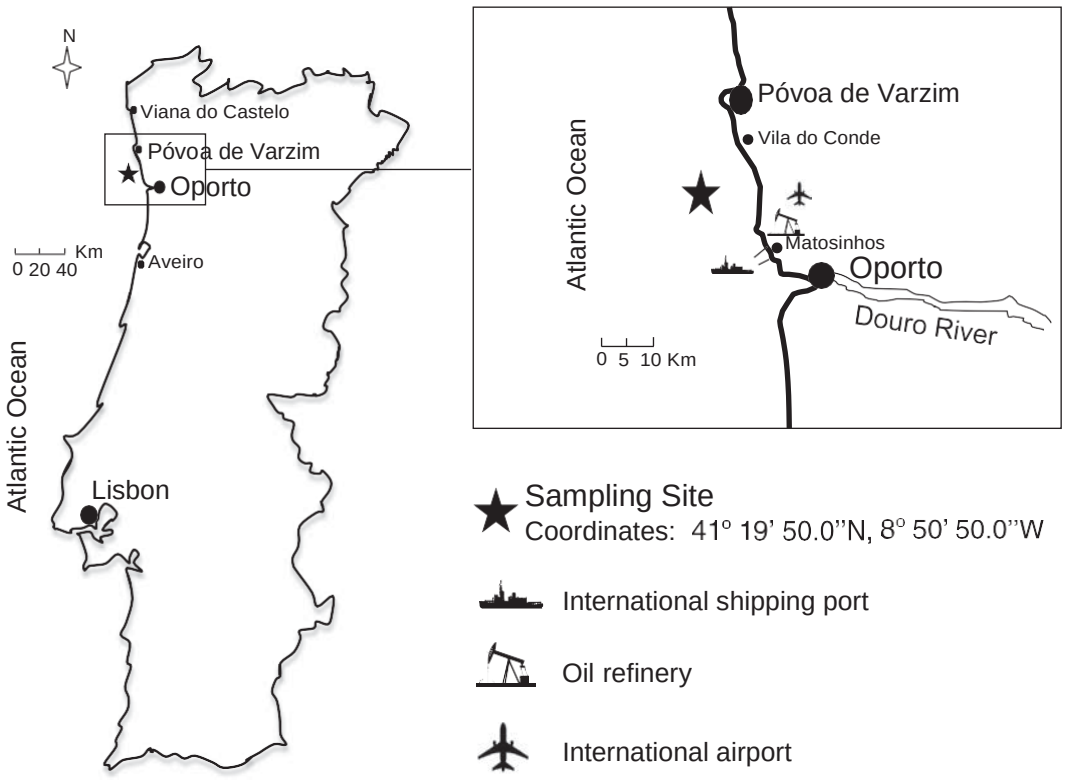

Fig. 1. Geographical location of octopus capture. Samplings were made in January, June, November 2011, and September 2012.

at 4 °C. The pellet was then suspended in ice-cold 100 mM potassium phosphate buffer with 20% v/v glycerol pH 7.4. Microsomal suspension (10 μL), with average protein concentration of 1.93 ± 0.27 mg/mL, was incubated with ethoxyresorufin (2 μM) for 1 min, and the enzymatic reaction initiated by the addition of NADPH 45 μM. EROD activity was measured for 20 min (λ_{ex} = 530 nm and λ_{em} = 585 nm) and determined by comparison to a resorufin standard curve. EROD activity is expressed in pmol/min/mg protein. For the measurement of ECOD activity 20 μL of the microsomal suspension were incubated with ethoxycoumarin (1 mM) for 1 min, and the enzymatic reaction initiated by the addition of NADPH 45 μM. ECOD activity was measured for 20 min (λ_{ex} = 380 nm and λ_{em} = 452 nm) and determined by comparison to a hydroxycoumarin standard curve. Protein content in the microsomal fractions was measured by the Lowry method using bovine serum albumin (BSA) as a standard (Lowry et al., 1951).

2.4. PAHs quantification

Microwave-assisted extractions of PAHs from octopus tissues were performed accordingly with some previous studies of the team (Gomes et al., 2013; Ramalhosa et al., 2012a, 2012b) with a MARS-X 1500 W (Microwave Accelerated Reaction System for Extraction and Digestion, CEM, Matthews, NC, USA) configured with a 14 position carousel. Briefly, extractions were performed at the optimal conditions: quartz extraction vessels, 1.3 g of arm or 0.5 g of digestive gland, 20 min at 110 °C with 20 mL of acetonitrile and medium stirring speed. A cleanup step with Sep-Pack Silica Plus cartridge (Waters) was performed for the extracts of the digestive glands. The extracts were then reduced to a small volume using a rotary evaporator (Buchi Rotavapor, R-200) at 20 °C. Then, a gentle stream of nitrogen was used to evaporate the extracts and immediately before chromatographic analysis, the residue was redissolved in 1.0 mL of acetonitrile. Extracts were analyzed using a LC system (Shimadzu Corporation, Kyoto, Japan) equipped with a LC-20AB pump (high-pressure gradient solvent delivery module equipped with two dual-plunger tandem-flow pumps), DGU-20AS degasser and photodiode array SPD-M20A (PAD) and fluorescence RF-10AXL (FLD) detectors on line (Gomes et al., 2013; Ramalhosa et al., 2012a, 2012b). Separation of the compounds was performed in a C18 column (CC 150/4 Nucleosil 100-5 C18 PAH, 150 × 4.0 mm; 5 μm particle size; Macherey-Nagel, Duren, Germany). The initial composition of the mobile phase was 50% of acetonitrile and 50% water and a linear gradient to 100% was programmed in 15 min, with a final hold of 13 min. Initial conditions were reached in 1 min and maintained for 6 min before next run. Fluorescence wavelength programming was used to perform better sensitivity and minimal interference. Each PAH was detected at its optimum excitation/emission wavelength pair (Gomes et al., 2013; Ramalhosa et al., 2012a, 2012b): 260/315 nm (naphthalene, acenaphthene and fluorene), 260/366 nm (phenanthrene), 260/430 nm (anthracene, fluoranthene, pyrene, benz(a)anthracene, crysene, benzo (b + j)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz (a,h)anthracene, benzo(g,h,i)perylene and dibenzo(a,l)pyrene and fluoranthene/pyrene(Fln/Pyr) was applied) and 290/505 nm (indeno (1,2,3-cd)pyrene). Acenaphthylene, which shows limited fluorescence, was analyzed at 254 nm in PAD. The overall MAE-LC procedure for analysis of PAHs was previously validated by analyzing the certified reference material SRM 2977 (mussel tissue) (Ramalhosa et al., 2012b) and by systematic recovery experiments at four spiking levels using the sample matrix. Recoveries ranged from 65.0 ± 3.0% (phenanthrene) to 101.2 ± 4.2% (pyrene). The attained detection and quantification limits were, respectively, 0.27 and 0.91 μg/kg wet weight (w.w.) for naphthalene, 8.1 and 27.1 μg/kg w.w. for acenaphthylene, 0.28 and 0.92 μg/kg w.w. for acenaphthene, 0.06 and 0.20 μg/kg w.w. for fluoranthene, 0.05 and 0.16 μg/kg w.w. for pyrene, 0.11 and 0.37 μg/kg w.w. for benzo(b)fluoranthene, 0.046 and 0.15 μg/kg w.w. for benzo(g,h,i) perylene, 0.04 and 0.12 μg/kg w.w. for fluorene, phenanthrene, anthracene, benzo(k)fluoranthene, chrysene, benz(a)anthracene, benzo(a) pyrene, dibenz(a,h)anthracene, and indeno(1,2,3-cd)pyrene. External calibrations with PAHs mixed matrix matched standards using 6 calibration points were performed (Gomes et al., 2013). Analytical blanks and PAHs mixed matrix matched standards were included in each

batch of samples to check instrument performance. Each analysis was run at least in triplicate. The carcinogenicity of PAH mixtures can be evaluated by calculating the total toxic benzo(a)pyrene equivalent (TEQ_{BaP}) that uses the toxicity equivalency factors (TEF) reported by various authors (Muller et al., 1997; Nisbet and LaGoy, 1992; Okona-Mensah et al., 2005). This approach uses benzo(a)pyrene as the index compound (having a relative potency of 1.0) and includes TEF values for seventeen PAHs assigning relative potencies in comparison to benzo(a)pyrene toxicity. The calculations to assess carcinogenic potential in the arm of octopus tissues were made according to the following equation $TEQ_{BaP} = \Sigma C_n \times TEF_n$ where C_n is the concentration of the individual PAH in the sample and TEF_n is the toxicity equivalency factor for that compound.

2.5. Statistical analysis

Differences between groups (captivity period and season) were tested using two-way ANOVA with a Tukey HSD test at 5% significance level. Some data were log transformed to fit ANOVA assumptions of normality and homogeneity of variance (Σ PAH concentrations, naphthalene, pyrene and chrysene concentrations in the digestive gland; % of fluorene, phenanthrene, pyrene, benzo(a)anthracene and two-ring PAHs in the digestive gland; Σ PAH concentrations, fluorene and phenanthrene concentrations in the arm; % of naphthalene, fluorene, phenanthrene and three-ring PAHs in the arm). Correlations were tested by Pearson analysis. Samples with PAH levels lower than the limit of detection were not used for statistical analysis. All statistical tests were performed using Statistica 7.0 (StatSoft Inc.).

2.6. Ethics statement

All animals were treated in accordance with the Portuguese Animals and Welfare Law (Decreto-Lei no. 113/2013) approved by the Portuguese Parliament in 2013 and with the European directive 2010/63/UE approved by the European Parliament in 2010 (Parliament, 2010). Institutional approval by CIIMAR/UP and General Veterinary Direction was granted for animals used in this study.

3. Results

3.1. Patterns in digestive gland of O. vulgaris

3.1.1. Phase I biotransformation enzymes

In January we were able to detect phase I biotransformation activity in the octopus digestive gland by means of EROD activity. In this period mean EROD activity was 0.66 ± 0.09 pmol/min/mg protein at day 0 and decreased to undetectable levels at day 14. No ECOD activity was detected in octopus digestive gland in January. In the other three sampling periods neither EROD nor ECOD activities were detected in the octopus digestive gland.

3.1.2. PAH levels

Individual and total PAH concentrations in the digestive gland of octopus are presented in Table 2. At the day 0, the concentrations of 18 PAHs in the digestive gland ranged from 10.6 to 103.7 μg/kg w.w. (mean of 36.7 μg/kg w.w.) being similar to the levels detected at day 14 where the range was 5.1 to 81.0 μg/kg w.w. (mean of 33.6 μg/kg w.w.).

Table 2
Mean, range and total (Σ) PAH concentrations (μg/kg w.w.) in the digestive gland of octopus collected in the four sampling periods.

		January		June		November		September	
	Rings	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
Naphthalene	2	9.98 (4.01-19.5)	16.7 (4.41-30.5)	12.5 (3.43-25.0)	5.58 (0.36-14.9)	27.1 (13.4-43.4)	51.6 (26.9-77.7)	19.1 (10.4-23.4)	7.44 (7.14-7.73)
Acenaphthylene	3	n.d.	14.2 (n.d.-14.2) 1.80	17.2 (n.d.-17.2) 2.68	n.d.	n.d.	49.9 (n.d.-49.9)	17.3 (n.d.-26.4) 0.96	20.8 (n.d.-28.5) 2.15
Acenaphthene	3	2.29 (n.d.-2.29)	(n.d.-1.80)	(n.d.-2.68)	4.84 (n.d.-4.84)	n.d.	n.d.	(n.d.-1.31)	(n.d.-3.62)
Fluorene	3	1.87 (n.d.-5.11)	2.57 (n.d.-2.81)	1.90 (0.08-7.29)	0.55 (0.14-0.85)	3.96 (0.21-28.3)	0.34 (0.29-0.39)	0.12 (0.09-0.15)	0.26 (0.24-0.29)
Phenanthrene	3	2.73 (2.17-3.69)	3.76 (2.94-4.95)	1.25 (n.d.-2.34)	2.26 (1.36-3.63)	13.6 (0.38-72.9)	0.73 (n.d.-2.51)	1.34 (n.d.-2.32)	n.d.
Anthracene	3	0.11 (n.d.-0.11)	0.08 (n.d.-0.08)	0.28 (n.d.-0.75)	0.29 (n.d.-0.44)	1.41 (n.d.-3.07)	1.71 (n.d.-2.71)	0.30 (n.d.-0.51)	0.42 (n.d.-0.72)
Fluoranthene	4	1.02 (0.79-1.24)	0.85 (0.77-0.96)	1.42 (n.d.-6.61)	0.22 (n.d.-0.30)	1.53 (n.d.-3.51)	1.08 (n.d.-1.70)	n.d.	0.36 (n.d.-0.36)
Pyrene	4	1.49 (0.74-3.28)	2.56 (n.d.-3.47)	0.58 (n.d.-1.04)	0.99 (0.80-1.17)	3.16 (n.d.-5.59)	0.79 (n.d.-1.47)	0.37 (n.d.-0.59)	0.43(n.d.-0.46)
Benz(a)anthracene	4	0.73 (0.45-1.03)	0.61 (0.39-0.85)	1.42 (0.04-2.85)	0.83 (0.32-1.77)	0.66 (0.20-1.43)	0.64 (n.d.-1.07)	0.78 (n.d.-0.78)	0.31 (n.d.-0.31)
Chrysene	4	0.76 (0.20-1.39)	0.25 (nd.-0.47)	1.72 (n.d.-5.02)	0.72 (n.d.-2.97)	0.91 (n.d.-2.40)	0.47 (n.d.-0.47)	7.66 (n.d.-24.9)	8.60 (n.d.-13.5)
Benzo(b + j)fluoranthene	5	0.56 (n.d.-0.60)	0.56 (n.d.-0.56)	0.56 (n.d.-0.91)	0.50 (n.d.-0.81)	2.46 (n.d.-4.71)	0.52 (n.d.-0.52)	n.d.	n.d.
Benzo(k)fluoranthene	5	0.08 (0.07-0.09)	n.d.	0.59 (0.04-3.19)	0.05 (n.d.-0.05)	0.10 (n.d.-0.17)	0.04 (n.d.-0.04)	n.d.	n.d.
Benzo(a)pyrene	5	n.d	n.d.	0.13 (n.d.-0.33) 0.29	0.11 (n.d.-0.11)	1.23 (n.d.-2.32)	0.14 (n.d.-0.17)	n.d.	n.d.
Dibenzo(a,l)pyrene	6	n.d.	n.d.	(n.d.-0.29)	n.d.	n.d.	n.d.	n.d.	n.d.
Dibenz(a,h)anthracene	5	0.78 (n.d.-1.05)	n.d.	n.d.	n.d.	2.30 (n.d.-2.95)	0.93 (n.d.-0.93)	1.41 (n.d.-1.41)	n.d.
Benzo(g,h,i)perylene	6	0.20 (n.d.-0.26)	0.13 (n.d.-0.13)	0.36 (n.d.-0.49)	0.23 (0.12-0.40)	2.09 (n.d.-2.09)	n.d.	n.d.	n.d.
Indeno(1,2,3-cd)pyrene	6	0.18 (n.d.-0.19) 19.8	n.d.	1.04 (n.d.-1.04) 22.6	0.26 (n.d.-0.26) 12.4	2.71 (n.d.-4.65) 53.9	n.d.	n.d.	n.d.
Σ PAHs		(17.0-26.0)	29.5 (10.5-39.4)	(10.6-41.5)	(5.1-23.3)	(24.4-103.7)	64.8 (47.0-81.0)	32.8 (18.6-51.2)	29.5 (25.9-36.5)

n.d. − not detected.

The most abundant PAH in the octopus digestive gland in the four studied periods was naphthalene that reached overall mean concentrations of 18.2 ± 9.6 μg/kg w.w. at day 0 accounting for about 50% of the total PAHs (from 47.0% in January to 63.6% in September). Generally, at day 0, the contribution of low molecular weight PAHs (LMW – 2-3 rings) for the Σ PAHs was about 75% of Σ PAHs (from 72.6% in January to 83.1% in November), while 4 ring PAHs accounted for about 20% (10.4% in November to 21.3% in January), 5 ring PAHs represented 4% (from 0.7% in September to 4.8% in January) and 6 ring PAHs represented only about 1% (from 0.0% in September to 2.1% in November) of Σ PAHs (Table 3).

Seasonal variability was detected in the Σ PAHs concentration in the digestive gland at day 0 (Fig. 2A). The highest values of Σ PAHs were found in November (autumn) being significantly higher ($p < 0.05$) than levels found in January and June. This increase was mainly due to the contribution of naphthalene, that triplicated from January to November, and the sharp increase in concentrations of phenanthrene, anthracene, pyrene and benzo(b + j)fluoranthene observed in November (Table 2).

In the four periods studied, the 14 day captivity did not have a significant ($p < 0.05$) effect on the Σ PAH concentrations in the digestive gland (Fig. 2A). Nevertheless, some changes were detected in particular groups of PAHs for each season. In January, all the 5 and 6 ring PAH concentrations decreased (in some cases to n.d. levels) after the 14 days while 2, 3 and 4 ring PAHs maintained or even increased concentrations (Table 2). The relative percentage (% of Σ PAHs) of compounds with 5 and 6 rings decreased more than 10 times after captivity in this period (Table 3). In June, all groups of PAHs (2-6 rings) exhibited lower concentrations after captivity being significant ($p < 0.05$) the decrease in naphthalene to half the amount detected at day 0 (Table 2). Indeed, the relative amount of naphthalene decreased from 50 to 30% (Table 3). Following this decrease, compounds with 3, 4 and 6 rings increased their percentage after 14 days (Table 3). The reduction observed in acenaphthylene from 17.2 μg/kg w.w. at day 0 to undetected levels at day 14 is not significant since it was detected in only one individual at day 0. The same decrease, as in January, in the relative and absolute amounts of 5 and 6 ring PAHs was observed in November with a concomitant increase in naphthalene concentration (Tables 2, 3). A remarkable decrease in phenanthrene concentration (from 13.6 ± 25.8 to 0.73 ± 1.19 μg/kg) was observed in November after 14 days (Table 2). However, this decrease was not statistically significant probably due to the high variability observed which is commonly found in field studies concerning PAH concentrations (Francioni et al., 2007). September was atypical in comparison to the other sampling periods and only PAHs with 2-4 rings were detected in a representative number of specimens (Tables 2, 3).

3.1.3. PAH source analysis

One approach to estimate the origin of PAH mixtures is to use the concentration ratios based on the thermodynamic stability of isomeric compounds (Francioni et al., 2007; Karacık et al., 2009; Webster et al., 2002). In this study, the simultaneous association of two concentration ratios, Phenanthrene/Anthracene (Phe/Ant) and Fluoranthene/Pyrene,(Fln/Pyr) was applied (Soclo et al., 2000; Webster et al., 2002) (Fig. 3). Phenanthrene and pyrene are more thermodynamically stable than the respective isomers anthracene and fluoranthene, causing a higher proportion of these compounds if the source is petrogenic (Webster et al., 2002). Pyrolysis of organic matter at high temperature generates a PAH mixture characterized by a low Phe/Ant ratio (< 10) and a high Fln/Pyr ratio (> 1.0) (Fig. 3) while lower temperature processes cause larger values of Phe/Ant ratio and lower values of Fln/Pyr ratio (Karacık et al., 2009; Webster et al., 2002). The ratios for the mean concentrations of these PAHs in the digestive gland are plotted in Fig. 3. In January (Phe/Ant = 25.1, Fln/Pyr = 0.7), petrogenic inputs predominated while in June (Phe/Ant = 4.4, Fln/Pyr = 2.4) both ratios indicate the major contribution of pyrolytic sources. November (Phe/Ant = 9.7, Fln/Pyr = 0.5) and September (Phe/Ant = 4.4, Fln/Pyr = 0.9) appear to show mixed contamination sources.

3.2. Patterns in arm of O. vulgaris

3.2.1. PAH levels

Individual and total PAH concentrations in the arm of octopus are presented in Table 4. At day 0, the concentrations of the 18 PAHs in the arm ranged from 0.07 to 31.3 μg/kg w.w. (mean of 6.13 μg/kg w.w.) being higher than levels found at day 14 where the range was 0.49 to 5.44 μg/kg w.w. (mean of 2.54 μg/kg w.w.).

In the arm, the most abundant PAHs varied throughout the year: in January naphthalene represented about 60% of Σ PAHs, in June and November acenaphthene corresponded to ca. 60% of Σ PAHs, and in September phenanthrene accounted for 70% of Σ PAHs. Generally, at day 0, the LMW PAHs represented more than 75% of Σ PAHs (being 95.4% in September), 4 ring PAHs drastically changed along the year (decreasing from 23.8% in January to 0.0% in September) while PAHs with 5 rings were never above 0.2% and 6 ring PAHs oscillated between 0.9 and 9.0% of Σ PAHs (Table 5) in November and June respectively.

Seasonal variability was also found in the Σ PAH concentrations in the arm (Fig. 2B) at day 0. The highest levels of total PAHs were found in June being significantly ($p < 0.05$) higher than those determined in November (3-4 times) and September (50 times higher). The main contributors for the high levels detected in June were acenaphthene (undetected levels in January, or September, to mean concentrations of 10.7 ± 4.7 μg/kg w.w. in June) and benzo(g,h,i)perylene (mean of 3.92 ± 8.11 μg/kg w.w. ca. 10 times higher than the concentrations found in the other sampling periods where it was found in only one – November and September – or two individuals – January) (Table 4).

In the four periods studied, the only significant difference in the Σ PAH concentrations in the arm after captivity was registered in June where the Σ PAHs decreased 10 times after 14 days ($p < 0.05$) (Fig. 2B). This decrease was detectable in almost all the individual PAHs (Table 4). The major reductions were observed in acenaphthene

Table 3
Mean and range of the relative amounts (% of Σ PAHs), grouped by number of rings, in the digestive gland of octopus collected in the four sampling periods.

		January		June		November		September	
	Rings	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
% of Σ PAHs	2	47.0 (23.0-74.9)	54.1 (28.7-77.6)	54.8 (32.3-80.4)	32.4 (6.2-64.2)	58.4 (12.9-83.6)	81.7 (34.4-98.3)	63.6 (39.8-93.0)	26.0 (19.6-29.4)
	3	25.6 (10.7-43.3)	30.1 (15.7-59.4)	20.8 (4.1-47.4)	37.1 (8.9-50.4)	24.7 (1.7-73.5)	15.8 (0.4-65.4)	15.8 (0.3-56.2)	50.2 (17.7-79.2)
	4	21.3 (11.1-38.2)	15.4 (4.6-29.3)	19.4 (4.9-40.2)	24.5 (11.3-36.3)	10.4 (5.1-20.0)	2.1 (0.0-3.9)	20.0 (1.1-59.3)	23.9 (1.3-53.4)
	5	4.8 (2.2-9.8)	0.4 (0.0-1.4)	3.7 (0.0-16.5)	2.5 (0.0-8.1)	4.4 (0.0-13.5)	0.5 (0.0-1.8)	0.7 (0.0-4.1)	–
	6	1.2 (0.0-2.4)	0.1 (0.0-0.4)	1.2 (0.0-4.6)	3.5 (0.8-11.4)	2.1 (0.8-13.1)	–	–	–

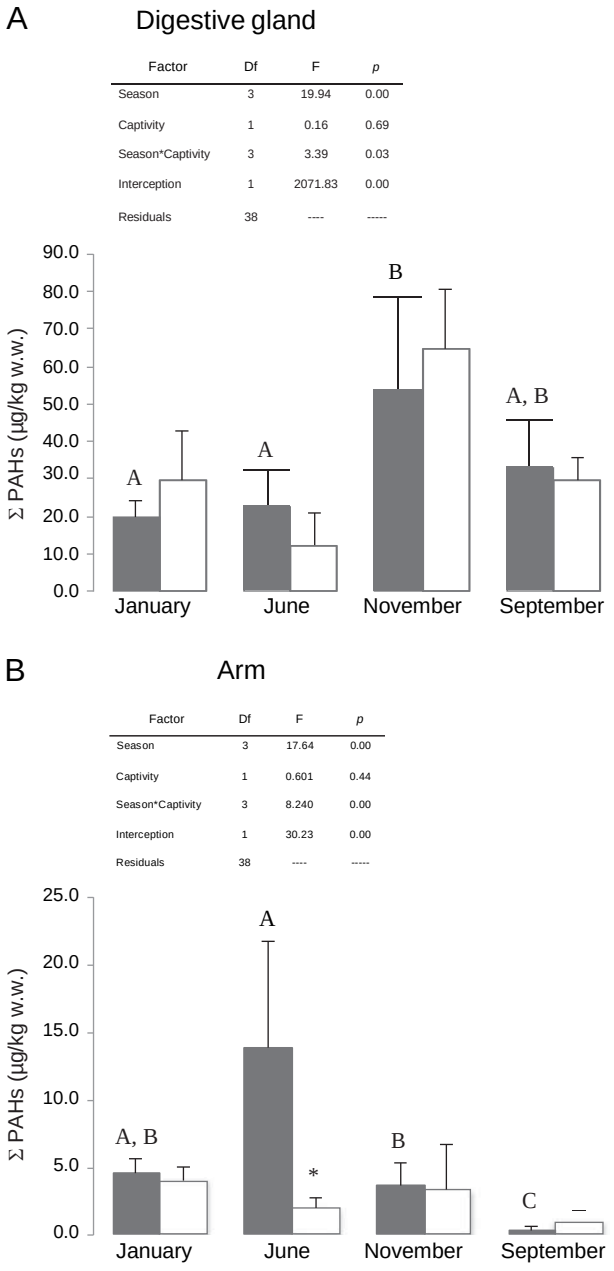

Fig. 2. Σ PAHs concentration in the (A) digestive gland and (B) arm of octopus at day 0 (●) and at day 14 (□) in January, June, November and September. Values are presented as mean ± SD. Different letters indicate significant differences (p < 0.05) between sampling periods at day 0. Significant differences between day 0 and day 14 for each sampling period are marked with * (p < 0.05). Tables with the two-way ANOVA results for the Σ PAHs (log transformed) are also presented for each tissue (Df – degrees of freedom).

(from 10.7 ± 4.7 µg/kg w.w. to undetected levels) and benzo(g,h,i) perylene (from 3.92 ± 8.11 to 0.18 ± 0.03 µg/kg w.w.) concentrations. Furthermore, the concentrations of the three ring PAHs decreased after captivity in this period which caused the greater change in the PAHs profile after 14 days of all the periods studied (Tables 4, 5). Despite these changes in June, in the other three periods no significant changes were detected neither in absolute nor relative concentrations of any PAHs after 14 days in captivity.

3.2.2. Carcinogenic PAH levels

Benzo(a)pyrene (group 1 – carcinogenic to humans) was detected in the arm of only one individual with 0.08 µg/kg w.w. (Table 4). PAHs classified as group 2A (probable carcinogens) were not detected in

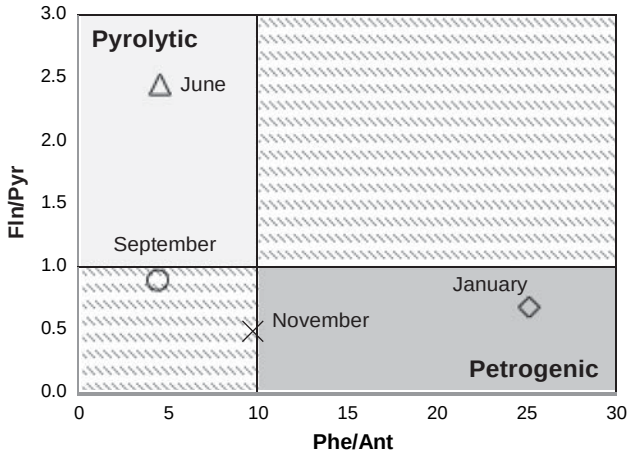

Fig. 3. Plot of the fluoranthene/pyrene (Fln/Pyr) ratio by the phenanthrene/anthracene (phe/ant) ratio in the digestive gland of *Octopus vulgaris* considering the PAH's source.

any specimen. The PAHs classified as group 2B (possible carcinogenic to humans), naphthalene, benz(a)anthracene, chrysene, benzo(b + j) fluoranthene, and benzo(k)fluoranthene, were detected in the arm in some of the sampling periods (Table 4). The means of the sum of the concentrations of these PAHs ($\Sigma_{carcinogenic}$ PAHs) for each period at day 0 and day 14 are also presented in Table 4. The global mean levels observed at day 0 for $\Sigma_{carcinogenic}$ PAHs in the arm varied from 2.50 µg/kg w.w. in January to undetectable levels in September. Calculated levels of TEQ_{BaP} for the four sampling periods are presented in Table 6. The maximum levels of TEQ_{BaP} were observed in June (45.90 and 31.88 ng/kg w.w. at day 0 and day 14 respectively). The major individual PAH contributing for the TEQ_{BaP} values changed throughout the year, being naphthalene in January (48%), benzo(g,h,i)perylene in June (51%) and September (70%), and acenaphthene in November (48%). In June and November, the 14 days captivity led to a minor decrease in the TEQ_{BaP} profile (Table 6).

4. Discussion

4.1. Patterns in digestive gland of O. vulgaris

4.1.1. Phase I biotransformation enzymes

This study evaluated PAH seasonal patterns in digestive gland and arm of octopus (*O. vulgaris*). In addition, detoxification ability of this species was assessed in captivity in controlled conditions. Negligible levels of EROD activity were found in January and undetectable levels for the rest of the year, both at day 0 and at day 14. These results were in accordance with previous studies in other octopus species (*Octopus pallidus*) that reported low levels of EROD activity in the digestive gland (Butty and Holdway, 1997; Cheah et al., 1995). Indeed, low levels of EROD activity have been observed for marine invertebrates which are possibly due to less responsive or different biotransformation pathways than the ones of vertebrates (Butty and Holdway, 1997; Cheah et al., 1995; Livingstone, 1998). However, the absence of ECOD activity was not expected and might be due to low method sensitivity or to a lower pollution pressure in the area where *O. vulgaris* was captured when compared to the sampling area of *O. pallidus* (Butty and Holdway, 1997; Cheah et al., 1995; Phillips et al., 1992). Thus, more research is needed to unravel the detoxification pathways adopted by this species to cope with the presence of contaminants in the environment.

4.1.2. PAH levels

In the present study, the levels of total PAHs found in the digestive gland of octopus were similar to previous results reported in mussel tissues from the NW Portuguese coast (Lima et al., 2008; Santos et al., 2010). Moreover, PAH levels in the digestive gland were below the

Table 4
Mean, range and total (Σ) PAH concentrations (μg/kg w.w.) in the arm of octopus collected in the four sampling periods.

		January		June		November		September	
	Rings	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
Naphthalene	2	2.50 (2.22-2.65)	2.41 (1.92-3.02)	1.61 (n.d.-2.71)	n.d.	0.98 (n.d.-0.98)	0.97 (n.d.-0.97)	n.d.	n.d.
Acenaphthylene	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acenaphthene	3	n.d.	n.d.	10.7 (n.d.-18.4)	n.d.	3.24 (n.d.-5.10)	3.22 (n.d.-3.22)	n.d.	n.d.
Fluorene	3	0.23 (0.19-0.34)	0.30 (0.17-0.48)	0.46 (0.10-1.04)	0.12 (n.d.-0.12)	0.33 (n.d.-1.10)	0.20 (n.d.-0.42)	0.18 (n.d.-0.30)	0.61 (0.29-0.83)
Phenanthrene	3	0.44 (0.32-0.69)	0.33 (0.28-0.37)	1.53 (0.10-6.25)	0.58 (0.38-0.98)	0.48 (0.11-0.78)	0.40 (0.19-0.56)	0.13 (0.05-0.23)	0.21 (0.17-0.26)
Anthracene	3	n.d.	0.08 (n.d.-0.08)	0.10 (n.d.-0.12)	0.06 (n.d.-0.06)	0.16 (n.d.-0.17)	n.d.	n.d.	n.d.
Fluoranthene	4	1.49 (n.d.-2.08)	0.39 (n.d.-0.59)	0.29 (n.d.-0.41)	0.42 (n.d.-1.00)	0.24 (n.d.-0.35)	1.06 (n.d.-1.06)	n.d.	n.d.
Pyrene	4	0.12 (n.d.-0.16)	0.95 (n.d.-1.75)	0.74 (n.d.-1.82)	0.35 (0.11-0.77)	0.26 (n.d.-0.30)	0.20 (n.d.-0.20)	n.d.	n.d.
Benz(a)anthracene	4	n.d.	0.23 (n.d.-0.23)	0.61 (n.d.-1.46)	0.11 (n.d.-0.16)	0.10 (n.d.-0.10)	n.d.	n.d.	n.d.
Chrysene	4	n.d.	n.d.	0.38 (n.d.-0.45)	0.21 (0.15-0.28)	0.16 (n.d.-0.16)	0.39 (n.d.-0.39)	n.d.	n.d.
Benzo(b + j)fluoranthene	5	n.d.	n.d.	0.22 (n.d.-0.22)	0.45 (n.d.-0.45)	n.d.	n.d.	n.d.	n.d.
Benzo(k)fluoranthene	5	n.d.	n.d.	n.d.	0.04 (n.d.-0.04)	0.04 (n.d.-0.04)	n.d.	n.d.	n.d.
Benzo(a)pyrene	5	n.d.	n.d.	n.d.	0.08 (n.d.-0.08)	n.d.	n.d.	n.d.	n.d.
Dibenzo(a,l)pyrene	6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Dibenz(a,h)anthracene	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzo(g,h,i)perylene	6	0.15 (n.d.-0.18)	0.21 (n.d.-0.21)	3.92 (n.d.-20.4)	0.18 (0.13-0.22)	0.24 (n.d.-0.24)	0.23 (n.d.-0.23)	0.20 (n.d.-0.20)	0.19 (n.d.-0.19)
Indeno(1,2,3-cd)pyrene	6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ PAHs		4.43 (3.32-5.46)	3.95 (3.22-5.44)	13.6 (4.60-31.3)	1.84 (1.02-3.16)	3.54 (0.11-6.21)	1.78 (0.57-3.90)	0.25 (0.07-0.73)	0.89 (0.49-1.17)
$\Sigma_{carcinogenic}$ PAHs		2.50 (2.22-2.65)	2.47 (2.15-3.02)	1.24 (0.00-3.16)	0.34 (0.15-0.80)	0.17 (0.00-1.03)	0.27 (0.00-1.36)	n.d.	n.d.

n.d. – not detected.

levels detected in mussels from Galician waters 2-3 months after the *Prestige* oil spill or in mussels from some "hot spots" such as the Galician Rias (concentrations of total PAHs above 500 μg/kg dry weight) (Soriano et al., 2006). In comparison to other cephalopod species the total PAH concentrations found in the digestive gland are overall higher than in the whole body of squids (Gomes et al., 2013) and deep-sea squids (over 1000 m depth) from the western Atlantic Ocean (Unger et al., 2008), nevertheless these differences are not higher than 3 folds. These results suggest that the exposure to PAHs in the NW Portuguese coast is moderate; however it must be taken into account that octopuses used in this study were captured around 10 km offshore and the contamination levels decrease with increasing distance from the pollution sources (Baumard et al., 1999). The high metabolism of PAHs in fish might have also contributed to the low levels found in the octopus digestive gland since Osteichthya species makes up a great part of the octopus diet in the NW Portuguese coast (Rosa et al., 2004a).

The PAH profile observed, with a predominance of LMW PAHs over HMW PAHs, is frequently reported in marine organisms (Gomes et al., 2013; Ramalhosa et al., 2012a; Perugini et al., 2007). As referred above, the octopus diet in the NW Portuguese coast is constituted mainly of Osteichthya species (Rosa et al., 2004a) that directly absorb PAHs from the water column, supporting the greater contribution of LMW than HMW PAHs observed in the digestive gland of the octopus (Nakata et al., 2003; Perugini et al., 2007; Webster et al., 2002). Naphthalene was the most abundant PAH in the octopus digestive gland in the different sampling periods. Many monitoring studies concerning PAH pollution do not quantify this 2-ring compound (Frias et al., 2010; Lima et al., 2008; Perugini et al., 2007; Santos et al., 2010; Soclo et al., 2000; Soriano et al., 2006) even though it is considered a possible carcinogenic to humans according to IARC (IARC, 2010).

Total PAH concentrations in the octopus digestive gland exhibited a maximum in November (autumn). Higher levels of PAHs in mussel

Table 5
Mean and range of the relative amounts (% of Σ PAHs), grouped by number of rings, in the arm of octopus collected in the four sampling periods.

		January		June		November		September	
	Rings	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
% of Σ PAHs	2	58.9 (47.5-78.4)	64.7 (35.2-80.4)	11.4 (0.0-59.0)	–	3.7 (0.0-29.9)	11.7 (0.0-58.6)	–	–
	3	15.3 (11.2-19.4)	17.2 (12.0-24.1)	72.5 (22.3-100)	34.2 (23.8-43.2)	89.9 (53.2-100)	67.3 (17.5-100)	95.4 (72.4-100)	94.5 (83.5-100)
	4	23.8 (0.0-38.1)	17.2 (0.0-47.2)	6.8 (0.00-26.4)	51.3 (37.8-64.4)	5.3 (0.0-11.2)	18.5 (0.0-68.5)	–	–
	5	–	–	0.2 (0.0-1.8)	3.2 (0.0-15.6)	0.2 (0.0-1.3)	–	–	–
	6	1.9 (0.0-5.5)	0.9 (0.0-3.8)	9.0 (0.0-65.3)	11.3 (6.1-19.0)	0.9 (0.0-7.2)	2.5 (0.0-12.3)	4.6 (0.0-27.6)	5.5 (0.0-16.5)

Table 6
TEF-adjusted mean concentrations of PAHs in the arm of octopus collected in the four sampling periods (ng/kg w.w.).

		January		June		November		September	
PAH	TEF	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
Naphthalene	0.001[a]	2.50	2.42	0.96	–	0.12	0.19	–	–
Acenaphthylene	0.001[a]	–	–	–	–	–	–	–	–
Acenaphthene	0.001[a]	–	–	7.51	–	2.43	0.64	–	–
Fluorene	0.001[a]	0.23	0.30	0.45	0.02	0.20	0.16	0.09	0.61
Phenanthrene	0.00064[b]	0.28	0.21	0.98	0.37	0.30	0.26	0.08	0.13
Anthracene	0.01 [a]	–	0.21	0.31	0.10	0.40	–	–	–
Fluoranthene	0.001[a]	1.12	0.29	0.15	0.35	0.06	0.21	–	–
Pyrene	0.001[a]	0.06	0.48	0.37	0.35	0.13	0.04	–	–
Benz[a]anthracene	0.014[b]	–	0.80	2.56	0.75	0.34	–	–	–
Chrysene	0.026[b]	–	–	1.98	5.34	0.51	2.05	–	–
Benzo[b]fluoranthene	0.11[b]	–	–	2.42	8.28	–	–	–	–
Benzo[k]fluoranthene	0.037[b]	–	–	–	0.26	0.19	–	–	–
Benzo[a]pyrene	1[a,b]	–	–	–	13.89	–	–	–	–
Dibenzo[a.l]pyrene	100 [c]	–	–	–	–	–	–	–	–
Dibenzo[a,h]anthracene	0.89[b]	–	–	–	–	–	–	–	–
Benzo[g,h,i]perylene	0.012[b]	0.92	0.62	28.19	2.16	0.36	0.54	0.40	0.77
Indeno[1,2,3-c,d]pyrene	0.067[b]	–	–	–	–	–	–	–	–
TEQ_{BaP} (ng/kg ww)	–	5.11	5.33	45.90	31.88	5.06	4.10	0.57	1.52

[a] Nisbet and LaGoy (1992).
[b] Muller (1997).
[c] Okona-Mensah et al. (2005).

tissues and of PAH metabolites in fish bile (biomarkers of exposure to PAHs) were previously detected in the autumn season in the same area (EROCIPS Environmental Monitoring Report; http://www.arcopol.eu, November, 2013) in agreement with the results of this study. Seasonal variability in PAH levels in tissues has been reported in several studies associated with an increase in the exposure or/and linked to biological factors such as the reproductive cycle of the organisms (Baumard et al., 1999; Webster et al., 2002). Considering that November follows the season where the fat availability in the Portuguese coast is higher (Bandarra et al., 1997, 2001), associated with a rise in sea temperature that favors higher rates of food intake (Mangold and Boletzky, 1973), the higher concentrations of Σ PAHs are expected due to their lipophilicity. Previous studies reported no evidence of energy reserves and lipid transfer between the digestive gland and the gonad with the reproductive status in octopus so this factor was probably not influencing the PAH concentrations observed (Quetglas et al., 2011; Rosa et al., 2004b), but instead, fat availability and food intake were influential.

The captivity in controlled conditions did not have a significant effect on the total PAHs concentration in the digestive gland. The low levels of PAHs detected and the low biotransformation capacity of this species, in comparison to vertebrate species, can explain this lack of PAH elimination after 14 days (Butty and Holdway, 1997; Ferreira et al., 2004; Livingstone, 1998). Still some changes after captivity were observed in particular groups of PAHs. An apparent selectivity towards the reduction of heavier PAHs (4–6 rings) rather than lighter PAHs (2–3 rings) was observed mainly in January and November. In June, this pattern of variation was not detected. The depuration effects on the PAH profile in June were significantly different since the main reduction was exhibited in the naphthalene (2 rings) amount.

4.1.3. PAH source analysis

In this study, the emission sources of PAHs were investigated in the digestive gland of octopus. This knowledge is of great importance to track the origin and fate of toxic compounds in the environment and supplies a good tool for taking administrative actions concerning environmental pollution (Wolska et al., 2011). Seasonal variability was observed in the emission sources of PAHs, a petrogenic profile was observed in January while in June a pyrolytic pattern emerged. Petrogenic PAHs are usually emitted directly to a water body whereas pyrolytic are first emitted into the air (Tobiszewski and Namieśnik, 2012). The sampling site is located near urban centers being the most important PAH pollution sources, the vehicle traffic, an oil refinery and a petrochemical complex, a power plant, an incineration unit, an international shipping port and airport (Slezakova et al., 2013). The highest precipitation in the months before January may have increased the river and urban runoff of petrogenic PAHs in this period (www.ipma.pt, June, 2013). In spring months, such as June, the higher traffic intensity (planes, cruises, etc.) possibly favored the atmospheric inputs of combustion derived PAHs thus a predominant pyrolytic source was identified in spring. Several authors concluded that the atmosphere is an important vector for PAH inputs to the Mediterranean waters, atmospheric inputs being higher than riverine inputs in some basins (Castro-Jiménez et al., 2012; Tsapakis et al., 2006; Tsapakis and Stephanou, 2005). Diagnostic PAH ratios applied to sediments from diverse geographical areas (Gulf of Gdansk; El-Menofiya governorate in Egypt; in the lagoon system Orbetello, Central Italy; Persian Gulf; coast of Aliaga, Turkey; Izmir Bay, Turkey; in Esquimalt and Victoria, BC, Canada; Abu Qir Bay, Egyptian Mediterranean Sea; western coast of Alexandria, Egypt) have also shown that deposition of compounds produced by combustion are one of the main sources (Nasr et al., 2010; Neşer et al., 2012; Rogowska et al., 2010; Wolska et al., 2011; Yunker et al., 2012; Zhang et al., 2012). September and November appear to show mixed contamination sources which might be caused by superimposing petrogenic and pyrolytic sources at the sampling site. These might also have been due to the high individual variability found and the uncertainty given by the low PAH concentrations found. It is important to notice that these results are indications of possible main emission sources but further evaluation of environmental PAHs concentration, for example in the sediments, would contribute to elucidate these results.

4.2. Patterns in arm of O. vulgaris

4.2.1. PAH levels

The levels of total PAHs in the octopus arm were lower (below one order of magnitude) than in the digestive gland as it happened with other contaminants in this species, e.g. metals (Raimundo et al., 2010; Semedo et al., 2012). The digestive gland is rich in fatty acids, which explains its major potential for bioaccumulation of PAHs which are lipophilic compounds. The levels of total PAHs in the arm observed in the present work are similar to the ones found in the edible parts of other cephalopod (squid) species captured in the Adriatic Sea and also in samples acquired in Catalonia markets (Martí-Cid et al., 2008; Perugini et al.,

2007). Moreover, concentrations determined in the common octopus in our study were below the total PAH concentrations found in the edible parts of two squid species from the Atlantic Ocean bought in markets of the NW region of Portugal (Gomes et al., 2013).

Seasonal variability in the total PAH concentrations in the arm was found in this study. By descending order, the highest mean of total PAH concentrations in the arm was found in June, January, November and September. In a similar way as reported in a previous study (Gomes et al., 2013) with squids, this series corresponds also to the descending order of the weight of the octopus sampled (Table 1). Indeed, total PAH concentrations in the arm were positively correlated with the weight ($r = 0.50$, $p < 0.05$). Usually weight is proportional to the age of the specimens so these results suggest the possibility of PAH bioaccumulation in the arm of the common octopus (Perales-Raya et al., 2010). Further research is undergoing since the influence of biometric parameters on PAH bioaccumulation is far from being consensual in seafood (Akpambang et al., 2009; Gomes et al., 2013; Perugini et al., 2007; Ramalhosa et al., 2012a; van der Oost et al., 2003). However, no information was found related with octopus species.

4.2.2. Carcinogenic PAH levels

In this study, benzo(a)pyrene, the only PAH classified by IARC as group 1 (known carcinogenic to humans) (IARC, 2010) was detected in the arm of only one individual with levels about 10 times below the maximum limit, 5 μg/kg w.w., previously established by the EC Regulation for cephalopods without viscera (Commission E., 2006). PAHs classified by IARC as type 2B possible carcinogenic to humans (naphthalene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(j) fluoranthene, benzo(k)fluoranthene) were also determined in the arm but at low amounts comparing to the most recent regulatory limits (12.0–30 μg/kg w.w. Commission, 2011) for the sum of benzo(a) pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene concentrations in seafood. The results obtained are in accordance with previous studies with cephalopod species from the Atlantic Ocean (Gomes et al., 2013), the Catalonia markets (Martí-Cid et al., 2008) and the Adriatic Sea (Perugini et al., 2007). The period with higher carcinogenic PAH concentrations was January. However, the carcinogenicity of the PAH mixture evaluated by using the TEQ_{BaP} approach revealed June as the period with higher toxicity levels. Nevertheless, the TEQ_{BaP} values obtained for all the sampling periods in the edible tissues of octopus captured in the NW Portuguese coast were markedly lower than the ones found in various aquatic species from different marine environments (Karacık et al., 2009; Perugini et al., 2007). In terms of PAH contamination, these results point out that there are no health risks for human moderate consumption of the octopus captured in the area.

5. Conclusions

Our study revealed that PAHs in the digestive gland of *O. vulgaris* exhibit seasonality in the amounts detected and in their main emission sources. Despite these seasonal variations along the year, general low levels of PAHs were detected in the digestive gland and in the arm. A general lack of PAH elimination by *O. vulgaris* was observed during captivity which is possibly explained by the low levels of PAHs detected and an apparently low biotransformation capacity of this species. Generally, octopus does not appear as a good sentinel organism for PAH monitoring. The levels of PAHs in the octopus arm (edible part) were generally an order magnitude lower than in the digestive gland and the carcinogenic PAHs, when detected, were most of the time more than an order of magnitude lower than the regulatory limits for food consumption. This study concerning an ecological and economical relevant species gave important baseline data for environmental monitoring of organic pollution in coastal areas. Further studies are being conducted in order to get some more insights about the PAH detoxification ability of *O. vulgaris* since available information is far from comprehensive.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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